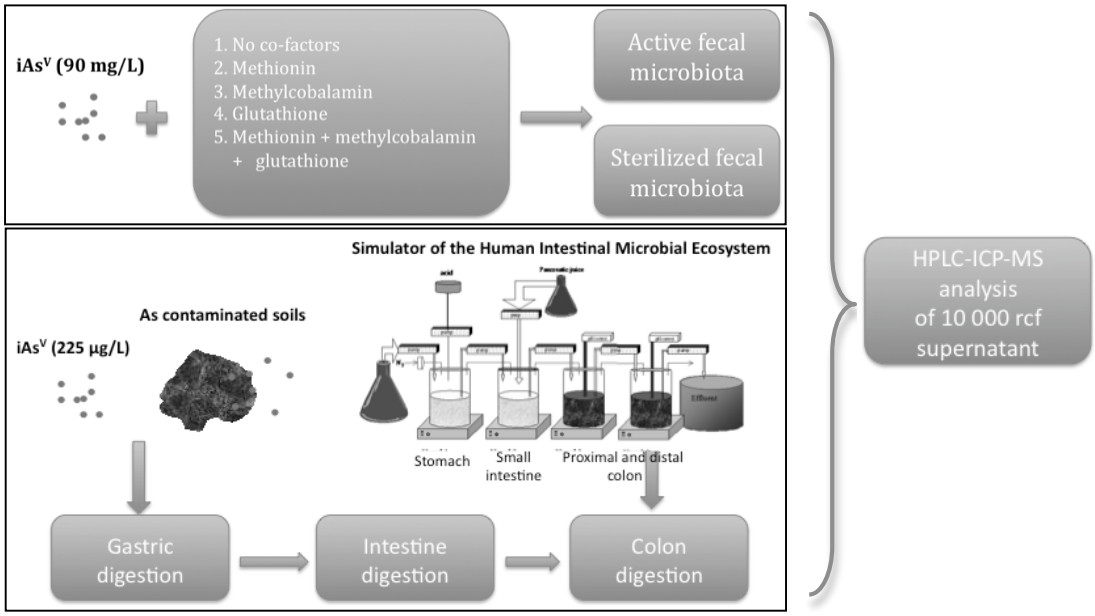
Supplemental information

Overview of batch experiments

The purpose of the first experiment was to screen the metabolic potency of fecal microorganisms towards high levels of iAs^V (90 mg/L). In addition, the influence from putative co-factors towards microbial arsenic metabolism was assessed. An overview of the screening experiment is depicted in Supplementary Figure 1.



Suppl. Figure 1. Schematic representation of the incubation experiments with fecal microbiota (top) and colon microbiota (bottom)

Standard control of the in vitro *cultured colon microbial community*

Operating a simulator of the human intestinal microbial ecosystem (SHIME) consists of the inoculation of a fecal microbial community from a human individual into the colon compartments. After a stabilization period of typically three weeks, a distinctive microbial community develops in each of the colon compartments. There is always a certain degree of dynamics and biological variation in the start-up period of such a SHIME model. Therefore, the *in vitro* cultured microbial community residing in the colon compartments must meet standard criteria in terms of microbial metabolic

activity and community composition. These are then typically compared to values from literature and the validation study of the SHIME model (Molly et al. 1994). The production of short chain fatty acids as a result of carbohydrate fermentation is the first important benchmark. Evaluation of short chain fatty acid concentrations in the distal colon compartment, from which suspension was sampled to perform the arsenic batch incubations, showed normal values of around 3000 mg/L (suppl. Table 1). In addition, the composition of the colon microbial community was evaluated. Both the total level of anaerobes and aerobes as that of specific microbial groups such as Bifidobacteria, Lactobacilli, Enterococci and Clostridia were found to be similar to those from previous studies. After steady-state of the microbial community was achieved in the different colon compartments, microbial suspension was sampled from the distal colon compartment to perform batch incubation experiments with iAs^{V} at 225 µg/L and arsenic contaminated soils (Supplementary Figure 1).

Supplementary Table 1. Fermentation activity (short chain fatty acid production and ammonium production) and microbial community composition of the distal colon compartment of the SHIME reactor.

Fermentation activity	mg/L	Community composition	log cfu/mL
Acetate	1633	Total anaerobes	8,7
Propionate	720	Total aerobes	7,4
Butyrate	474	Lactobacilli	5,2
Branched chain fatty acids	140	Bifidobacteria	6,9
Total short chain fatty acids	2967	Enterococci	6,2
Ammonium	392	Clostridia	7,0

Arsenic sulfides: species synthesis, identification and sample analysis

A mixture of arsenic standards of mono, di, and trithioarsenate (AsO_3S^{III}, $AsO_2S_2^{III}$, and $AsOS_3^{III}$, respectively) were synthesized according to Schwedt et al. (1996). The thioarsenate mixture was analyzed by using a PRP-X100 with 10mM ammonium phosphate and 10mM ammonium nitrate with 500ppm EDTA mobile phase. AsO_3S^{III} eluted at 15 min and $AsO_2S_2^{III}$ eluted at 45 min, while $AsOS_3^{III}$ did not elute within the

separation window. The sample and a sample spiked with a standard thioarsenate mixture were analyzed and the unknown peaks were tentatively identified as AsO_3S^{III} and $AsO_2S_2^{III}$. To further confirm the identity, a secondary separation based on work by Wallschlager et al. (2007) using a IonPac AS-16 (4 x 250 mm, 5 µm, Dionex, Sunnyvale, CA) with a mobile phase of 2.7% (w/w) tetramethylammonium hydroxide (TMAOH) in DIW at a flow rate of 1.0 mL min-1 (50 µL were injected). The sample and a sample spiked with a standard thioarsenate mixture were analyzed and the two unknown peaks from the first separation were confirmed as AsO_3S^{III} and $AsO_2S_2^{III}$.

Additional confirmation of $MMMTA^{V}$ was carried out using a second chromatographic method with both ICP-MS and ESI-MS (Thermo LTQ, Thermo Scientific). The HPLC separation utilized a Phenomenex C_{18} column (250 x 4.6 mm, 5µm) with a mobile phase of 15 mM $(NH_4)_2CO_3$, 10% methanol (w/w) at pH 4.6 (adjusted with acetic acid) in DDI. The flow rate was 0.5 mL/min and the injection volume was 25 µL (**Separation 2**). Under these conditions, $MMMTA^{V}$ eluted at 7.5 min. The flow from the HPLC was split 1:1 between the ICP-MS and the ESI-MS. The ESI-MS was operated in negative ion mode with a source voltage of 3.5 kV, source current of 100 µA, sheath gas flow of 70, auxiliary gas flow of 20, and capillary temperature of 250°C. The molecular ion of $MMMTA^{V}$ was fragmented in MS/MS mode by collisionally activated dissociation.

Chromatographic recovery

Arsenic speciation analysis in this study relied on chromatographic separation with HPLC and subsequent detection with ICP-MS. However, it is possible that the microbial metabolism of arsenic results in the formation of metabolites that do not elute from the chromatographic column and, for this reason, go undetected via this method. A mass balance between the total arsenic and the chromatographable arsenicals was calculated to assess the chromatographic recovery of the method. If this parameter is approximately 100%, it indicates that all the arsenicals present in

the sample are eluting from the chromatographic column. If this parameter is very low, a large amount of unchromatographable As species may be present. In the latter case, it is difficult to draw conclusions from bioaccessibility values or rates of microbial metabolism. The chromatographic recoveries for colon digests of iAs^V and soil 1, 2, 3 and 4 are given in Suppl. Table 2. Except for the colon digest of soil 4, the slag soil, all colon digests displayed a satisfactorily high chromatographic recovery: 108, 90, 95 and 83% for the colon digests of iAs^V, soil 1, soil 2 and soil 3, respectively. These values led us to conclude that the HPLC-ICP-MS method was reliable to obtain a realistic view of arsenic speciation changes upon colon digestion.

Supplementary Table 2. Chromatographic recoveries for colon digests incubated with pure iAs^V and As contaminated soils. Values are represented as averages (n=3) ± the standard deviation.

	Chromatographic recovery[a] (%)
iAs^V	108 ± 39
Soil 1	90 ± 21
Soil 2	95 ± 15
Soil 3	83 ± 11
Soil 4	13 ± 3

[a] Chromatographic recovery was calculated by dividing the sum of chromatographically detected (HPLC-ICP-MS) arsenic species (iAs^V, iAs^{III}, MMA^V, MMA^{III} and $MMMTA^V$) in the colon digest supernatants by the total amount of arsenic in the colon digest supernatants as measured by ICP-OES.

References for supplemental information

Molly K, Vandewoestyne M, Desmet I, Verstraete W. 1994. Microbial Ecology in Health and Disease 7: 191-200.

Schwedt G, Rieckhoff M. 1996. Separation of thio- and oxothioarsenates by capillary zone electrophoresis and ion chromatography. Journal of Chromatography A 736: 341-350.

Wallschlager D, Stadey CJ. 2007. Determination of (oxy)thioarsenates in sulfidic waters. Analytical Chemistry 79: 3873-3880